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(54) **Use of an enzyme for assisting an animal to digest protien**

(57) The present invention provides the use of a xylanase for assisting livestock to digest protein and/or amino acids present in a feed. Such a use increases the protein and amino acid digestibility of the livestock's diet. Alternatively, such a use enables the actual protein content of a feed to be reduced by including lower levels of relatively costly protein supplements such as fishmeal and meatmeal. The use also enables the content of energy supplements present in the feed to be reduced from the amounts conventionally used without reducing the feed's nutritional value. Preferably the feed comprises a cereal and the animal is a chicken.

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# USE OF AN ENZYME FOR ASSISTING AN ANIMAL TO DIGEST PROTEIN

The present invention relates to the use of an enzyme for assisting an animal to digest protein, and in particular for enabling livestock to digest protein and/or amino acids present in their feed more efficiently. The invention also provides a method for increasing the energy and/or protein and/or amino acid value of the cereal component of a feed.

Improvements in animal feeds to enable animals to digest them more efficiently are constantly being sought. One of the main concerns is to improve the Feed Conversion Ratio (FCR) of a feed without increasing its cost per unit weight. The FCR of a feed is the ratio of the amount of feed consumed relative to the weight gain of the animal. A low FCR indicates that a given amount of feed results in a growing animal gaining proportionately more weight. This means that the animal is able to utilize the feed more efficiently. One way in which the FCR of a feed can be reduced is to improve its digestibility by an animal thereby increasing the nutritional benefit which the animal can derive from it.

One of the key nutritional components of a feed is its protein fraction. It is a problem that pigs and poultry find cereals in particular to be deficient in their crude protein value and in the nutritional availability of amino acids which they contain. As a result, commercial cereal-based feeds have to be supplemented with additional protein sources and/or essential amino acids, such as lysine or methionine, in order to ensure that the feed has sufficient nutritionally available amounts of amino acids to provide livestock with a healthy diet.

The digestibility of protein and amino acids present in a feed by an animal is limited by:

(i) the viscosity of materials present in the animal's gut. Such viscosity is due, at least in part, to soluble non-starch polysaccharides such as mixed-linked ß-glucans and arabinoxylans;

(ii) entrapment of nutrients within the cell walls of the feed, particularly those of the aleurone layer in cereals. Such entrapment is caused by the high levels of polysaccharide in the cell walls of cereals which are relatively resistant to break-down by the animal's digestive system. This prevents nutrients entrapped within the cell walls from being nutritionally available to the animal; and

(iii) a deficiency in endogenous enzyme activity, both of the animal and of the gut microbial population particularly in a young animal.

It is already known to include enzyme supplements in animal feeds. For example, JP-A-60-75238 discloses a feed for domestic animals which contains an enzyme cocktail including protease-, cellulase-, amylase- and lipase-activities. This reference speculates that these various enzyme activities enable fermentation microbes to grow and these become useful nutritional components of the feed.

Can. J. Anim. Sci. 70: pp 623-633 notes that substituting naked oats for corn in a broiler starter diet reduces feed efficiency. This reduction in efficiency is countered by adding ß-D-glucanase to the diet which decreases ß-glucan viscosity in the gut which was a cause of the reduced feed efficiency.

It is an object of the present invention to provide the use of an enzyme for increasing the ability of an

animal to digest protein and/or amino acid present in its diet.

Accordingly, the present invention provides the use of a xylanase for assisting livestock to digest protein and/or amino acids present in a feed.

It has been found that the inclusion of a xylanase in the diet of an animal enables the animal to digest the protein and/or amino acid content of its diet more efficiently. Thus the addition of a xylanase to a feed increases the proportion of crude protein and/or amino acids which can be digested by the animal. As a result of this surprising finding, it is possible to modify conventional feeds by reducing their protein and/or amino acid content whilst simultaneously maintaining the same nutritional level of protein and/or amino acids available to the animal. This means that the amounts of costly protein supplements conventionally included in animal feed such as fishmeal, meatmeal, soybean, rapeseed or canola can be reduced as compared to conventional feeds. This results in a significant reduction in the cost per unit weight of the animal feed without decreasing its nutritional benefit. Alternatively, or even additionally, the amounts of amino acid supplements can be reduced as compared to conventional feeds which also can result in significant cost savings.

In a further aspect, the present invention provides a method for increasing the energy and/or protein and/or amino acid nutritional value of the cereal component of a cereal-based feed comprising the step of including 0.00001-10g of xylanase protein per kg of the feed.

In one further aspect,the present invention provides a method for enabling the amount of energy and/or

protein supplements and/or amino acid supplements included in a cereal-based feed to be reduced without reducing the feed's nutritional value comprising maintaining or increasing the cereal content of the feed whilst adding to it 0.00001-10g of xylanase per kg of the feed.

In a further aspect, the present invention provides a method for uplifting the energy and/or protein and/or amino acid value of a cereal-based feed or a component thereof comprising the step of including 0.00001-10g of xylanase protein per kg of the feed.

Such an addition of xylanase can raise the energy value of the cereal component of the feed by at least 6 %, preferably by at least 10%, and the protein and/or amino acid value of the cereal component of the feed by at least 10 %, preferably by at least 15%. Thus, it has been found that the controlled addition of a xylanase to a cereal-based feed increases its energy and/or protein and/or amino acids value which allows the amounts of costly energy and/or protein and/or amino acid supplements conventionally included to be reduced without affecting the overall performance of the feed. Thus not only does the presence of the xylanase have the beneficial effect of enabling the livestock to digest protein and/or amino acids more efficiently, it also aids in the digestion of costly energy supplements such as fats and oils.

The xylanase can be formulated as a pre-mix together with any other enzyme supplements to be included in the feed. The pre-mix can be added to the raw materials before feed manufacture, during feed manufacture or as a final step once the feed is otherwise ready for use. It is also possible to add the enzyme directly as a liquid to a feed material pre-formed as pellets or as a mash.

It is also possible to include the xylanase in the animal's diet by incorporating it into a second (and different) feed or drinking water which the animals also have access to. Accordingly, it is not essential that the xylanase is incorporated into the feed itself, although such incorporation forms a particularly preferred aspect of the present invention.

If the xylanase is incorporated into the feed, then this is preferably done in a relative amount of 0.00001-10 g of xylanase protein per kilo of the feed; more preferably 0.0001-1 g/kg; and most preferably 0.001-0.1 g/kg.

It is particularly preferred that the xylanase is included in a cereal-based feed. A cereal-based feed is one which includes at least 20% by weight of a cereal, and more preferably at least 30% by weight of the cereal, and most preferably at least 50% by weight of the cereal The cereal can be any one or more of wheat, rye, triticale, barley, oats, rice, sorghum and maize. It is however particularly preferred that the cereal is wheat.

Although the cereal component of a cereal-based diet constitutes a source of protein, it is usually necessary to include sources of supplementary protein in the diet such as those derived from fishmeal, meatmeal or vegetables. Sources of vegetable proteins include at least one of full fat soybeans, rapeseed, canola, soybean meal, rapeseed meal and canola meal. As compared to conventional feeds, the relative amount of the additional protein sources such as fishmeal, meatmeal or vegetable protein can be reduced by adopting the teaching of the present invention resulting in significant cost savings. This is because the relative cost of cereals is significantly less than that of conventional protein

supplements. In view of this, a feed can be prepared according to the present invention having the same nutritional value in terms of available energy, amino acids and protein as a conventional feed but which includes a higher relative proportion of cereal and a lower relative proportion of protein supplements.

As previously mentioned, it has also been found that the inclusion of a xylanase in an animal feed has the effect that reduced levels of energy supplements such as fats and oils need to be included in order to achieve a feed with a certain level of performance.

Accordingly, the addition of xylanase to an animal's diet, and in particular its feed, enables the crude protein value and/or digestibility and/or the amino acid content and/or digestibility coefficients of the diet to be increased, which permits a reduction in the amounts of alternative protein sources and/or amino acid supplements which have previously had to be included. When the protein digestibility coefficient and/or the content of available crude protein of wheat is increased by addition of xylanase, the major savings are to be found in the reduced levels of protein and/or energy supplements which need to be added. On the other hand, the levels of amino acid supplements used remain the same or may even have to be increased. However, on balance the cost per unit weight of the feed is reduced because of the relative expense of the alternative protein supplements such as soybean meal compared to cereal.

Alternatively, when only amino acid content or digestibility coefficient values are increased by the addition of xylanase, the major savings are to be found in the reduced levels of amino acid supplements which have conventionally needed to be added to the feeds.

The xylanase which is to be included in the livestock's diet can be obtained from any suitable source. For example, the xylanase may be obtained from a fungal source such as *Trichoderma, Aspergillus, Humicola or Neocallimastix*. It is particularly preferred that the xylanase is the low pI xylanase and/or the high pI xylanase obtainable from *Trichoderma longibrachiatum* such as described in WO 92/06209. It is also possible that the xylanase can be obtained from a bacterial species. It is also possible that the xylanase may be obtained from a host which has been subjected to genetic manipulation such as by the inclusion of an appropriate gene within a host bacterial or fungal strain.

The feed provided by the present invention may also include other enzyme supplements such as one or more of a ß-glucanase, protease, α-amylase and pectinase. It is particularly preferred to include a protease as an enzyme supplement such as subtilisin derived from the genus *Bacillus*. Such subtilisins are for example described in detail in US-A-4760025.

A suitable feed in accordance with the present invention can be obtained by preparing a pre-mix of xylanase, ß-glucanase and protease on a cereal carrier, and then adding 1 part by weight of this pre-mix to 1000 parts by weight of the animal feed including the cereal and other sources of protein supplement.

The present invention is further explained by way of the following Examples and Comparative Examples. Each of these examples relates to 42-day studies with broiler chickens fed with wheat-based diets. A starter feed is fed up to 21 days of age and subsequently a grower feed is fed from 21 to 42 days of age.

Comparative Example 1

Conventional wheat-based starter and grower feeds were respectively formulated in accordance with the following Tables 1 and 2.

Wheat-Based Starter

TABLE 1

| Ingredients | Percent | Cost | Weight |
|---|---|---|---|
| Soft wheat | 65.45% | 64.14 | 654.51 |
| Soybean ml 48 | 29.80% | 47.38 | 298.00 |
| Soy oil | 1.05% | 2.05 | 10.53 |
| Salt | 0.30% | 0.15 | 2.99 |
| DL Methionine | 0.22% | 5.42 | 2.21 |
| Lysine HCl | 0.05% | 1.16 | 0.51 |
| Limestone | 1.40% | 1.19 | 13.96 |
| Dicalcium Phos | 1.23% | 1.53 | 12.28 |
| VIT/MIN | 0.50% | 6.25 | 5.00 |
| | | | |
| TOTAL | 100.00% | $129.28 | 1000.00 |

Wheat-Based Grower

TABLE 2

| Ingredients | Percent | Cost | Weight |
|---|---|---|---|
| Soft wheat | 72.95% | 71.49 | 729.52 |
| Soybean ml 48 | 20.50% | 32.59 | 204.99 |
| Soy oil | 2.97% | 5.79 | 29.72 |
| Salt | 0.30% | 0.15 | 2.96 |
| DL Methionine | 0.07% | 1.68 | 0.69 |
| Lysine HCl | 0.07% | 1.55 | 0.69 |
| Limestone | 1.49% | 1.27 | 14.95 |
| Dicalcium Phos | 1.15% | 1.44 | 11.49 |
| VIT/MIN | 0.50% | 6.25 | 5.00 |
| | | | |
| TOTAL | 100.00% | $122.22 | 1000.00 |

The nutritional value of the above starter and grower feeds can be subjected to computer analysis using for example the programme "Format" available from Format International. This provides an analysis of the theoretical nutrient content of the feed including for example the expected levels of its various amino acids. The results of such analyses for the feeds of Tables 1 and 2 above are respectively set out in the following Tables 3 and 4.

## Wheat-Based Starter
### TABLE 3

| Nutrient | Target | Value | RQ? | %RQ |
|---|---|---|---|---|
| Crude protein | 22.50 | 22.50 | XXX | 100.00% |
| Poult ME kcal/ | 3000.00 | 3000.00 | XXX | 100.00% |
| Pig DE Kcal | | 3322.31 | | |
| Calcium % | 0.90 | 0.90 | XXX | 100.00% |
| Phos % | | 0.67 | | |
| Avail Phos % | 0.42 | 0.42 | | |
| Fat % | | 2.57 | | |
| Fibre % | | 2.62 | | |
| Met % | | 0.54 | | |
| Cys % | | 0.41 | | |
| Me+Cys % | 0.95 | 0.95 | XXX | 100.00% |
| Lys % | 1.20 | 1.20 | XXX | 100.00% |
| His % | | 0.54 | | |
| Tryp % | 0.24 | 0.29 | | |
| Thr % | 0.41 | 0.80 | | |
| Arg % | 0.85 | 1.47 | | |
| Iso % | | 1.02 | | |
| Leu % | | 1.64 | | |
| Phe % | | 1.13 | | |
| Val % | | 1.11 | | |
| Gly % | | 1.09 | | |
| Phe+Tyr % | | 1.86 | | |
| Na % | 0.15 | 0.15 | XXX | 100.00% |
| Cl % | | 0.25 | | |
| K % | | 0.89 | | |
| Linoleic acid | 1.00 | 1.08 | | |
| Na+K-Cl | | 2.23 | | |

## Wheat-Based Grower
### TABLE 4

| Nutrient | Target | Value | RQ? | %RQ |
|---|---|---|---|---|
| Crude protein | 19.00 | 19.00 | XXX | 100.00% |
| Poult ME kcal/ | 3175.00 | 3175.00 | | |
| Pig DE Kcal | | 3374.31 | | |
| Calcium % | 0.90 | 0.90 | XXX | 100.00% |
| Phos % | | 0.62 | | |
| Avail Phos % | 0.40 | 0.40 | | |
| Fat % | | 4.43 | | |
| Fibre % | | 2.48 | | |
| Met % | | 0.34 | | |
| Cys % | | 0.36 | | |
| Me+Cys % | 0.70 | 0.70 | XXX | 100.00% |
| Lys % | 0.95 | 0.95 | XXX | 100.00% |
| His % | | 0.45 | | |
| Tryp % | 0.19 | 0.24 | | |
| Thr % | 0.59 | 0.65 | | |
| Arg % | 0.95 | 1.17 | | |
| Iso % | | 0.83 | | |
| Leu % | | 1.36 | | |
| Phe % | | 0.95 | | |
| Val % | | 0.92 | | |
| Gly % | | 0.91 | | |
| Phe+Tyr % | | 1.54 | | |
| Na % | 0.15 | 0.15 | XXX | 100.00% |
| Cl % | | 0.25 | | |
| K % | | 0.72 | | |
| Linoleic acid | 1.00 | 1.89 | | |
| Na+K-Cl | | 1.79 | | |

Broiler chickens were fed with the wheat-based starter feed of Table 1 up to 21 days of age and then with the wheat-based grower feed from 21 to 42 days of age. The results are set out in Table 5 below.

## Example 1

The experimental routine of the above Comparative Example 1 was repeated except that both the wheat-based starter feed and the wheat-based grower feed were supplemented with an enzyme mix including inter alia xylanase. Thus, a pre-mix containing xylanase, ß-glucanase and protease was prepared and coated on a cereal carrier. This was then added to both of the above feeds in an amount of 1 part by weight per 1000 parts by weight of the feeds. The resulting feeds comprise about 0.0025 parts by weight of xylanase protein per 1000 parts by weight of the feeds. The results of this routine are set out in the following Table 5:

TABLE 5

| Example | Comparative Example 1 | Example 1 |
|---|---|---|
| Live weight gain, grammes | 2188 | 2177 |
| Feed Conversion Ratio (based on weight gain) | 1.644 | 1.613 |
| Average litter score | 3.250 | 3.375 |
| Hock lesions (%) | 56.67 | 41.67 |

From the results set out in Table 5, it can be seen that both Example 1 and Comparative Example 1 give rise to substantially the same gain in live weight. However, the FCR, based on weight gain, is significantly smaller for the composition of Example 1 as compared to Comparative Example 1. This indicates that feeds formulated in accordance with the present invention by including xylanase are utilised more efficiently than those of the prior art which do not include this enzyme.

The feeds of Example 1 also give rise to a reduced level of hock lesions caused by wet manure as compared to the feeds of the Comparative Example 1.

Comparative Example 2

Wheat-based starter and grower feeds were formulated respectively in accordance with Tables 6 and 7 below. Compared to the feeds used in Comparative Example 1 and Example 1, the relative amounts of wheat in these compositions were increased so that the wheat component of the feeds provides an assumed 15% increase both in its crude protein value and the amino acid digestibility coefficients for its wheat component. At the same time, the relative amounts of soybean meal were reduced compared with the feeds used in Comparative Example 1 and Example 1.

Wheat-Based Starter
TABLE 6

| Ingredients | Percent | Cost | Weight |
|---|---|---|---|
| Soft wheat | 69.14% | 67.76 | 691.40 |
| Soybean ml 48 | 26.33% | 39.23 | 263.30 |
| Soy oil | 0.79% | 1.54 | 7.90 |
| Salt | 0.30% | 0.15 | 3.00 |
| DL Methionine | 0.21% | 5.15 | 2.10 |
| Lysine HCl | 0.13% | 2.93 | 1.30 |
| Limestone | 1.45% | 1.23 | 14.50 |
| Dicalcium Phos | 1.14% | 1.43 | 11.40 |
| VIT/MIN | 0.51% | 6.38 | 5.10 |
| | | | |
| TOTAL | 100.00% | $125.78 | 1000.00 |

Wheat-Based Grower
TABLE 7

| Ingredients | Percent | Cost | Weight |
|---|---|---|---|
| Soft wheat | 77.71% | 76.16 | 777.12 |
| Soybean ml 48 | 16.24% | 25.82 | 162.39 |
| Soy oil | 2.43% | 4.75 | 24.34 |
| Salt | 0.29% | 0.15 | 2.94 |
| DL Methionine | 0.05% | 1.34 | 0.55 |
| Lysine HCl | 0.16% | 3.67 | 1.63 |
| Limestone | 1.56% | 1.33 | 15.62 |
| Dicalcium Phos | 1.04% | 1.30 | 10.41 |
| VIT/MIN | 0.50% | 6.25 | 5.00 |
| | | | |
| TOTAL | 100.00% | $120.76 | 1000.00 |

A computer analysis of the nutritional values of the above starter and grower compositions is set out respectively in the following Tables 8 and 9:

Wheat-Based Starter

TABLE 8

| Nutrient | Target | Value | RQ? | %RQ |
|---|---|---|---|---|
| Crude protein % | 22.50 | 22.59 | | |
| Poult ME kcal/kg | 3000.00 | 3009.81 | | |
| Pig DE Kcal | | 3302.55 | | |
| Calcium % | 0.90 | 0.89 | XXX | 99.42% |
| Phos % | | 0.64 | | |
| Avail Phos % | 0.42 | 0.40 | XXX | 95.83% |
| Fat % | | 2.35 | | |
| Fibre % | | 2.59 | | |
| Met % | | 0.53 | | |
| Cys % | | 0.42 | | |
| Me+Cys % | 0.95 | 0.95 | | |
| Lys % | 1.20 | 1.20 | | |
| His % | | 0.53 | | |
| Tryp % | 0.24 | 0.29 | | |
| Thr % | 0.41 | 0.79 | | |
| Arg % | 0.85 | 1.42 | | |
| Iso % | | 1.00 | | |
| Leu % | | 1.63 | | |
| Phe % | | 1.13 | | |
| Val % | | 1.10 | | |
| Gly % | | 1.09 | | |
| Phe+Tyr % | | 1.85 | | |
| Na % | 0.15 | 0.15 | | |
| Cl % | | 0.26 | | |
| K % | | 0.83 | | |
| Linoleic acid % | 1.00 | 1.01 | | |
| Na+K-Cl | | 2.04 | | |

Wheat-Based Grower

TABLE 9

| Nutrient | Target | Value | RQ? | %RQ |
|---|---|---|---|---|
| Crude protein | 19.00 | 19.00 | | |
| Poult ME kcal/ | 3175.00 | 3175.00 | | |
| Pig DE Kcal | | 3726.14 | | |
| Calcium % | 0.90 | 0.90 | XXX | 100.00% |
| Phos % | | 0.63 | | |
| Avail Phos % | 0.40 | 0.40 | | |
| Fat % | | 4.17 | | |
| Fibre % | | 2.74 | | |
| Met % | | 0.33 | | |
| Cys % | | 0.37 | | |
| Me+Cys % | 0.70 | 0.70 | | |
| Lys % | 0.95 | 0.95 | XXX | 100.00% |
| His % | | 0.44 | | |
| Tryp % | 0.19 | 0.23 | | |
| Thr % | 0.59 | 0.63 | | |
| Arg % | 0.95 | 1.12 | | |
| Iso % | | 0.80 | | |
| Leu % | | 1.33 | | |
| Phe % | | 0.94 | | |
| Val % | | 0.90 | | |
| Gly % | | 0.90 | | |
| Phe+Tyr % | | 1.53 | | |
| Na % | 0.15 | 0.15 | | |
| Cl % | | 0.27 | | |
| K % | | 0.65 | | |
| Linoleic acid | 1.00 | 1.71 | | |
| Na+K-Cl | | 1.56 | | |

Broiler chickens were fed with the starter and grower feeds according to the same experimental routine described in Comparative Example 1 above. The results are set out in Table 10 below.

Example 2

The experimental routine of the above Comparative Example 2 was repeated except that both the wheat-based starter and grower feeds were supplemented with an enzyme mix including inter alia xylanase. Thus, a pre-mix containing xylanase, β-glucanase and protease was prepared and coated on a cereal carrier. This was then added to both of the above feeds in an amount of 1 part by weight per 1000 parts by weight of the feeds. The resulting feed comprises about 0.0025 parts by weight of xylanase protein per 1000 parts by weight of the feed. The results of this routine are set out in the following Table 10:

TABLE 10

| Example | Comparative Example 2 | Example 2 |
|---|---|---|
| Live weight gain, grammes | 2036 | 2049 |
| Feed Conversion Ratio (based on weight gain) | 1.756 | 1.690 |
| Average litter score | 3.333 | 3.125 |
| Hock lesions (%) | 42.50 | 20.83 |

By comparing the results of Comparative Example 2 with those for Comparative Example 1 (Table 5), it can be seen that the FCR of the feed of the Comparative Example is substantially inferior demonstrating the effects of a comparatively reduced supply of protein and/or essential amino acids in the feeds of Comparative Example 2.

In the results set out in Table 10, the live weight gain is generally consistent within experimental error. However, the feeds used in Example 2 which include xylanase, give rise to a decreased and therefore a superior FCR as compared to feed compositions of Comparative Example 2 which lack xylanase. Further, the composition of the present invention also gives rise to a marked decrease in the incidence of hock lesions.

From the above results, it can be concluded that the broiler chickens lack protein and/or essential amino acids in Comparative Example 2, whereas protein and/or essential amino acid supply is substantially improved in the case of the feeds of Example 2. By including xylanase in the starter and grower feeds, the broiler chickens are able to digest proteins and amino acids present in the feeds more easily resulting in the feeds being perceived by the animal as possessing increased food protein value and having increased amino acid digestibility.

Comparing the diets of Examples 1 and 2, it can be seen that the amounts of soft-wheat contained in the feeds of Example 2 have been relatively increased whereas the amounts of soybean meal have been decreased. As a result, the overall costs of the compositions of Example 2 are lower than the costs of the corresponding feeds in Example 1. Nevertheless, the feeds used in Example 2 are commercially acceptable because the presence of xylanase enables the broiler chickens to more easily digest proteins and amino acids from the cereal content of the feeds than has previously been the case for feeds such as those used in Comparative Examples 1 and 2.

## Example 3

Wheat-based starter and grower feeds were formulated respectively in accordance with Tables 11-18 below. The feeds of Tables 11 and 15 are basic (control) starter and grower feeds. The feeds of Tables 12 and 16 are adjusted from those of Tables 11 and 15 by adding 6% to the apparent metabolisable energy (AME) value of the wheat component. The feeds of Tables 13 and 17 are adjusted from those of Tables 11 and 15 by adding 12% to the value of all amino acids of the

wheat component and 6% to the AME value of the wheat component. The feeds of Tables 14 and 18 are adjusted from those of Tables 11 and 15 by adding 12% to the crude protein value of the wheat component, 12% to the value of all amino acids of the wheat component, and 6% to the AME value of the wheat component.

**Wheat-based starter**
**Table 11**

| Ingredients | Percent | Cost | Weight |
|---|---|---|---|
| Soft wheat | 60.69% | 59.78 | 606.93 |
| Soybean ml 48 | 31.65% | 54:13 | 316.53 |
| Soy oil | 3.51% | 7.89 | 35.06 |
| Salt | 0.30% | 0.15 | 3.03 |
| DL Methionine | 0.22% | 4.97 | 2.16 |
| Limestone | 1.38% | 1.17 | 13.75 |
| Dicalcium Phos | 1.25% | 1.57 | 12.53 |
| VIT/MIN | 1.00% | 12.50 | 10.00 |
| | | | |
| TOTAL | 100.00% | £142.16 | 1000.00 |

**Wheat-based starter + 6% AME**
**Table 12**

| Ingredients | Percent | Cost | Weight |
|---|---|---|---|
| Soybean ml 48 | 30.08% | 51.44 | 300.84 |
| Soy oil | 1.34% | 3.03 | 13.45 |
| Wheat + 6 | 64.33% | 63.36 | 643.26 |
| Salt | 0.30% | 0.15 | 3.00 |
| DL Methionine | 0.32% | 7.32 | 3.18 |
| Limestone | 1.39% | 1.18 | 13.91 |
| Dicalcium Phos | 1.24% | 1.54 | 12.35 |
| VIT/MIN | 1.00% | 12.50 | 10.00 |
| | | | |
| TOTAL | 100.00% | £140.53 | 1000.00 |

**Wheat-based starter + 6٧ AME + 12٧ Amino Acids**
**Table 13**

| Ingredients | Percent | Cost | Weight |
|---|---|---|---|
| Soybean ml 48 | 30.08% | 51.44 | 300.84 |
| Soy oil | 1.34% | 3.03 | 13.45 |
| Wheat +6+12AA | 64.33% | 63.36 | 643.26 |
| Salt | 0.30% | 0.15 | 3.00 |
| DL Methionine | 0.32% | 7.32 | 3.18 |
| Limestone | 1.39% | 1.18 | 13.91 |
| Dicalcium Phos | 1.24% | 1.54 | 12.35 |
| VIT/MIN | 1.00% | 12.50 | 10.00 |
| | | | |
| TOTAL | 100.00% | £140.53 | 1000.00 |

**Wheat-based starter + 6% AME + 12% Amino Acids + 12% Crude Protein**
**Table 14**

| Ingredients | Percent | Cost | Weight |
|---|---|---|---|
| Soybean ml 48 | 27.34% | 46.75 | 273.38 |
| Soy oil | 1.01% | 2.27 | 10.08 |
| Wheat +6 +12CP/AA | 67.37% | 66.36 | 673.66 |
| Salt | 0.30% | 0.15 | 2.99 |
| DL Methionine | 0.35% | 8.03 | 3.49 |
| Limestone | 1.41% | 1.20 | 14.07 |
| Dicalcium Phos | 1.23% | 1.54 | 12.34 |
| VIT/MIN | 1.00% | 12.50 | 10.00 |
| | | | |
| TOTAL | 100.00% | £138.79 | 1000.00 |

**Wheat-based grower**
**Table 15**

| Ingredients | Percent | Cost | Weight |
|---|---|---|---|
| Soft wheat | 67.41% | 66.39 | 674.05 |
| Soybean ml 48 | 22.89% | 39.14 | 228.87 |
| Soy oil | 5.70% | 12.83 | 57.01 |
| Salt | 0.30% | 0.15 | 3.01 |
| DL Methionine | 0.06% | 1.36 | 0.59 |
| Limestone | 1.47% | 1.25 | 14.70 |
| Dicalcium Phos | 1.18% | 1.47 | 11.76 |
| VIT/MIN | 1.00% | 12.50 | 10.00 |
| | | | |
| TOTAL | 100.00% | £135.09 | 1000.00 |

**Wheat-based grower + 6% AME**
**Table 16**

| Ingredients | Percent | Cost | Weight |
|---|---|---|---|
| Soybean ml 48 | 22.60% | 38.65 | 226.05 |
| Soy oil | 3.49% | 7.85 | 34.87 |
| Wheat + 6 | 69.92% | 68.87 | 699.22 |
| Salt | 0.30% | 0.15 | 2.98 |
| DL Methionine | 0.05% | 1.19 | 0.52 |
| Limestone | 1.48% | 1.26 | 14.80 |
| Dicalcium Phos | 1.16% | 1.44 | 11.56 |
| VIT/MIN | 1.00% | 12.50 | 10.00 |
| | | | |
| TOTAL | 100.00% | £131.91 | 1000.00 |

**Wheat-based grower + 6 /o AME + 12% Amino Acids**
**Table 17**

| Ingredients | Percent | Cost | Weight |
|---|---|---|---|
| Soybean ml 48 | 25.41% | 43.46 | 254.13 |
| Soy oil | 3.83% | 8.61 | 38.25 |
| Wheat + 6 + 12AA | 66.72% | 65.71 | 667.15 |
| Salt | 0.30% | 0.15 | 3.00 |
| DL Methionine | 0.12% | 2.87 | 1.25 |
| Limestone | 1.46% | 1.24 | 14.63 |
| Dicalcium Phos | 1.16% | 1.45 | 11.58 |
| VIT/MIN | 1.00% | 12.50 | 10.00 |
| | | | |
| TOTAL | 100.00% | £135.99 | 1000.00 |

**Wheat-based grower + 6% AME + 12% Amino Acids + 12% Crude Protein**
**Table 18**

| Ingredients | Percent | Cost | Weight |
|---|---|---|---|
| Soybean ml 48 | 25.41% | 43.46 | 254.13 |
| Soy oil | 3.83% | 8.61 | 38.25 |
| Wheat + 6 + 12CP/AA | 66.72% | 65.71 | 667.15 |
| Salt | 0.30% | 0.15 | 3.00 |
| DL Methionine | 0.12% | 2.87 | 1.25 |
| Limestone | 1.46% | 1.24 | 14.63 |
| Dicalcium Phos | 1.16% | 1.45 | 11.58 |
| VIT/MIN | 1.00% | 12.50 | 10.00 |
| | | | |
| TOTAL | 100.00% | £135.99 | 1000.00 |

A computer analysis of the nutritional value of each of the above eight feed compositions using the "Format" computer programme is set out respectively in the following Tables 19-26.

**Wheat-based starter**
**Table 19**

| Nutrient | Target | Value | RQ? | %RQ |
|---|---|---|---|---|
| Crude protein % | 22.50 | 22.83 | | |
| Poult ME kcal/kg | 3000.00 | 3000.00 | XXX | 100.00% |
| Pig DE Kcal | | 3413.71 | | |
| Calcium % | 0.90 | 0.90 | XXX | 100.00% |
| Phos % | | 0.67 | | |
| Avail Phos % | 0.42 | 0.42 | XXX | 100.00% |
| Fat % | | 4.85 | | |
| Fibre % | | 2.56 | | |
| Met % | | 0.54 | | |
| Cys % | | 0.41 | | |
| Me+Cys % | 0.95 | 0.95 | | |
| Lys % | 1.20 | 1.20 | | |
| His % | | 0.55 | | |
| Tryp % | 0.24 | 0.30 | | |
| Thr % | 0.41 | 0.82 | | |
| Arg % | 0.85 | 1.51 | | |
| Iso % | | 1.05 | | |
| Leu % | | 1.67 | | |
| Phe % | | 1.14 | | |
| Val % | | 1.13 | | |
| Gly % | | 1.11 | | |
| Phe+Tyr % | | 1.89 | | |
| Na % | 0.15 | 0.15 | XXX | 100.00% |
| Cl % | | 0.24 | | |
| K % | | 0.91 | | |
| Linoleic acid % | 1.00 | 2.02 | | |
| Na+K-Cl | | 2.31 | | |

**Wheat-based starter + 6 ⁄₀ AME**
**Table 20**

| Nutrient | Target | Value | RQ? | %RQ |
|---|---|---|---|---|
| Crude protein % | 22.50 | 22.50 | XXX | 100.00% |
| Poult ME kcal/kg | 3000.00 | 3000.00 | XXX | 100.00% |
| Pig DE Kcal | | 3318.52 | | |
| Calcium % | 0.90 | 0.90 | XXX | 100.00% |
| Phos % | | 0.67 | | |
| Avail Phos % | 0.42 | 0.42 | XXX | 100.00% |
| Fat % | | 2.83 | | |
| Fibre % | | 2.60 | | |
| Met % | | 0.65 | | |
| Cys % | | 0.36 | | |
| Me+Cys % | 0.95 | 0.95 | XXX | 100.00% |
| Lys % | 1.20 | 1.27 | | |
| His % | | 0.61 | | |
| Tryp % | 0.24 | 0.40 | | |
| Thr % | 0.41 | 0.68 | | |
| Arg % | 0.85 | 1.35 | | |
| Iso % | | 1.16 | | |
| Leu % | | 1.44 | | |
| Phe % | | 1.32 | | |
| Val % | | 1.00 | | |
| Gly % | | 1.13 | | |
| Phe+Tyr % | | 1.65 | | |
| Na % | 0.15 | 0.15 | XXX | 100.00% |
| Cl % | | 0.24 | | |
| K % | | 0.89 | | |
| Linoleic acid % | 1.00 | 1.19 | | |
| Na+K-Cl | | 2.26 | | |

**Wheat-based starter + 6 ⁄ AME + 12% Amino Acids**
**Table 21**

| Nutrient | Target | Value | RQ? | %RQ |
|---|---|---|---|---|
| Crude protein % | 22.50 | 22.50 | XXX | 100.00% |
| Poult ME kcal/kg | 3000.00 | 3000.00 | XXX | 100.00% |
| Pig DE Kcal | | 3318.52 | | |
| Calcium % | 0.90 | 0.90 | XXX | 100.00% |
| Phos % | | 0.67 | | |
| Avail Phos % | 0.42 | 0.42 | XXX | 100.00% |
| Fat % | | 2.83 | | |
| Fibre % | | 2.60 | | |
| Met % | | 0.65 | | |
| Cys % | | 0.36 | | |
| Me+Cys % | 0.95 | 0.95 | XXX | 100.00% |
| Lys % | 1.20 | 1.27 | | |
| His % | | 0.61 | | |
| Tryp % | 0.24 | 0.40 | | |
| Thr % | 0.41 | 0.68 | | |
| Arg % | 0.85 | 1.35 | | |
| Iso % | | 1.16 | | |
| Leu % | | 1.44 | | |
| Phe % | | 1.32 | | |
| Val % | | 1.00 | | |
| Gly % | | 1.13 | | |
| Phe+Tyr % | | 1.65 | | |
| Na % | 0.15 | 0.15 | XXX | 100.00% |
| Cl % | | 0.24 | | |
| K % | | 0.89 | | |
| Linoleic acid % | 1.00 | 1.19 | | |
| Na+K-Cl | | 2.26 | | |

**Wheat-based starter + 6% AME + 12% Amino Acids + 12 % Crude Protein**
**Table 22**

| Nutrient | Target | Value | RQ? | %RQ |
|---|---|---|---|---|
| Crude protein % | 22.50 | 22.57 | | |
| Poult ME kcal/kg | 3000.00 | 3000.00 | XXX | 100.00% |
| Pig DE Kcal | | 3295.76 | | |
| Calcium % | 0.90 | 0.90 | XXX | 100.00% |
| Phos % | | 0.66 | | |
| Avail Phos % | 0.42 | 0.42 | XXX | 100.00% |
| Fat % | | 2.54 | | |
| Fibre % | | 2.58 | | |
| Met % | | 0.67 | | |
| Cys % | | 0.34 | | |
| Me+Cys % | 0.95 | 0.95 | | |
| Lys % | 1.20 | 1.20 | XXX | 100.00% |
| His % | | 0.59 | | |
| Tryp % | 0.24 | 0.39 | | |
| Thr % | 0.41 | 0.63 | | |
| Arg % | 0.85 | 1.26 | | |
| Iso % | | 1.11 | | |
| Leu % | | 1.35 | | |
| Phe % | | 1.29 | | |
| Val % | | 0.94 | | |
| Gly % | | 1.08 | | |
| Phe+Tyr % | | 1.56 | | |
| Na % | 0.15 | 0.15 | XXX | 100.00% |
| Cl % | | 0.24 | | |
| K % | | 0.85 | | |
| Linoleic acid % | 1.00 | 1.08 | | |
| Na+K-Cl | | 2.14 | | |

**Wheat-based grower**
**Table 23**

| Nutrient | Target | Value | RQ? | %RQ |
|---|---|---|---|---|
| Crude protein % | 19.00 | 19.49 | | |
| Poult ME kcal/kg | 3175.00 | 3175.00 | XXX | 100.00% |
| Pig DE Kcal | | 3478.77 | | |
| Calcium % | 0.90 | 0.90 | XXX | 100.00% |
| Phos % | | 0.62 | | |
| Avail Phos % | 0.40 | 0.40 | XXX | 100.00% |
| Fat % | | 6.96 | | |
| Fibre % | | 2.43 | | |
| Met % | | 0.34 | | |
| Cys % | | 0.36 | | |
| Me+Cys % | 0.70 | 0.70 | | |
| Lys % | 0.95 | 0.95 | | |
| His % | | 0.46 | | |
| Tryp % | 0.19 | 0.25 | | |
| Thr % | 0.59 | 0.68 | | |
| Arg % | 0.95 | 1.23 | | |
| Iso % | | 0.86 | | |
| Leu % | | 1.40 | | |
| Phe % | | 0.97 | | |
| Val % | | 0.94 | | |
| Gly % | | 0.94 | | |
| Phe+Tyr % | | 1.59 | | |
| Na % | 0.15 | 0.15 | XXX | 100.00% |
| Cl % | | 0.24 | | |
| K % | | 0.75 | | |
| Linoleic acid % | 1.00 | 2.93 | | |
| Na+K-Cl | | 1.90 | | |

**Wheat-based grower + 6 / AME**
**Table 24**

| Nutrient | Target | Value | RQ? | %RQ |
|---|---|---|---|---|
| Crude protein % | 19.00 | 19.64 | | |
| Poult ME kcal/kg | 3175.00 | 3175.00 | XXX | 100.00% |
| Pig DE Kcal | | 3385.07 | | |
| Calcium % | 0.90 | 0.90 | XXX | 100.00% |
| Phos % | | 0.63 | | |
| Avail Phos % | 0.40 | 0.40 | XXX | 100.00% |
| Fat % | | 4.89 | | |
| Fibre % | | 2.48 | | |
| Met % | | 0.33 | | |
| Cys % | | 0.37 | | |
| Me+Cys % | 0.70 | 0.70 | XXX | 100.00% |
| Lys % | 0.95 | 0.95 | XXX | 100.00% |
| His % | | 0.46 | | |
| Tryp % | 0.19 | 0.25 | | |
| Thr % | 0.59 | 0.68 | | |
| Arg % | 0.95 | 1.23 | | |
| Iso % | | 0.87 | | |
| Leu % | | 1.41 | | |
| Phe % | | 0.98 | | |
| Val % | | 0.95 | | |
| Gly % | | 0.95 | | |
| Phe+Tyr % | | 1.60 | | |
| Na % | 0.15 | 0.15 | XXX | 100.00% |
| Cl % | | 0.24 | | |
| K % | | 0.76 | | |
| Linoleic acid % | 1.00 | 2.07 | | |
| Na+K-Cl | | 1.90 | | |

**Wheat-based grower + 6% AME + 12 ⁄₀ Amino Acids**
**Table 25**

| Nutrient | Target | Value | RQ? | %RQ |
|---|---|---|---|---|
| Crude protein % | 19.00 | 20.59 | | |
| Poult ME kcal/kg | 3175.00 | 3175.00 | XXX | 100.00% |
| Pig DE Kcal | | 3408.40 | | |
| Calcium % | 0.90 | 0.90 | XXX | 100.00% |
| Phos % | | 0.63 | | |
| Avail Phos % | 0.40 | 0.40 | XXX | 100.00% |
| Fat % | | 5.19 | | |
| Fibre % | | 2.50 | | |
| Met % | | 0.43 | | |
| Cys % | | 0.33 | | |
| Me+Cys % | 0.70 | 0.70 | XXX | 100.00% |
| Lys % | 0.95 | 1.14 | | |
| His % | | 0.57 | | |
| Tryp % | 0.19 | 0.38 | | |
| Thr % | 0.59 | 0.59 | XXX | 100.00% |
| Arg % | 0.95 | 1.19 | | |
| Iso % | | 1.06 | | |
| Leu % | | 1.28 | | |
| Phe % | | 1.23 | | |
| Val % | | 0.89 | | |
| Gly % | | 1.03 | | |
| Phe+Tyr % | | 1.47 | | |
| Na % | 0.15 | 0.15 | XXX | 100.00% |
| Cl % | | 0.24 | | |
| K % | | 0.80 | | |
| Linoleic acid % | 1.00 | 2.18 | | |
| Na+K-Cl | | 2.03 | | |

**Wheat-based grower + 6% AME + 12% Amino Acids + 12 /o Crud Protein**
**Table 26**

| Nutrient | Target | Value | RQ? | %RQ |
|---|---|---|---|---|
| Crude protein % | 19.00 | 21.59 | | |
| Poult ME kcal/kg | 3175.00 | 3175.00 | XXX | 100.00% |
| Pig DE Kcal | | 3408.40 | | |
| Calcium % | 0.90 | 0.90 | XXX | 100.00% |
| Phos % | | 0.63 | | |
| Avail Phos % | 0.40 | 0.40 | XXX | 100.00% |
| Fat % | | 5.19 | | |
| Fibre % | | 2.50 | | |
| Met % | | 0.43 | | |
| Cys % | | 0.33 | | |
| Me+Cys % | 0.70 | 0.70 | | |
| Lys % | 0.95 | 1.14 | | |
| His % | | 0.57 | | |
| Tryp % | 0.19 | 0.38 | | |
| Thr % | 0.59 | 0.59 | XXX | 100.00% |
| Arg % | 0.95 | 1.19 | | |
| Iso % | | 1.06 | | |
| Leu % | | 1.28 | | |
| Phe % | | 1.23 | | |
| Val % | | 0.89 | | |
| Gly % | | 1.03 | | |
| Phe+Tyr % | | 1.47 | | |
| Na % | 0.15 | 0.15 | XXX | 100.00% |
| Cl % | | 0.24 | | |
| K % | | 0.80 | | |
| Linoleic acid % | 1.00 | 2.18 | | |
| Na+K-Cl | | 2.03 | | |

Groups of male Ross 1 broiler chickens were fed with one of the four wheat-based starter feeds of Tables 11-14 up to 21 days of age and then with the corresponding wheat-based grower feed of Tables 15-18 from 22 to 42 days of age. Sixty birds were included in each group, and each test was replicated nine times. For instance, the first-ninth groups, each of 60

birds, were fed the feed of Table 11 up to 21 days of age followed by the feed of Table 15 for days 22-42.

The above experimental routine was repeated except that both the wheat-based starter feed and the wheat-based grower feed of each of the four routines were supplemented with an enzyme mix including *inter alia* a xylanase. Thus a pre-mix containing a xylanase from *Trichoderma longibrachiatum* and a protease from *Bacillus sp.* was prepared and coated on a cereal carrier. This control cereal carrier was then added to both of the above feeds in an amount of one part by weight per 1000 parts by weight of the feeds. The resulting feeds comprise about 0.0025 parts by weight of xylanase protein per 1000 parts by weight of the feeds and 0.0003 parts by weight of protease protein per 1000 parts by weight of the feeds. The results of these eight separate routines are set out below in Tables 27 and 28.

**Table 27**

| Diet | Enzyme included | FCR | Gain, g | Feed Intake, g | Mortality % |
|---|---|---|---|---|---|
| Control (basic feed) | No | 1.428 | 649 | 986 | 1.68 |
| Control (basic feed) | Yes | 1.400 | 626 | 937 | 1.31 |
| +6% AME | No | 1.418 | 604 | 909 | 1.86 |
| +6% AME | Yes | 1.411 | 649 | 976 | 0.93 |
| +6% AME + 12%AA | No | 1.432 | 651 | 991 | 1.30 |
| +6% AME + 12%AA | Yes | 1.418 | 606 | 909 | 2.84 |
| +6% AME + 12%AA & CP | No | 1.447 | 585 | 905 | 1.68 |
| +6% AME + 12%AA & CP | Yes | 1.414 | 581 | 864 | 2.82 |

**Table 28**

| Diet | Enzyme included | FCR | Gain, g | Feed Intake, g | Mortality % |
|---|---|---|---|---|---|
| Control (basic feed) | No | 1.911 | 2104 | 3971 | 3.91 |
| Control (basic feed) | Yes | 1.843 | 2118 | 3861 | 4.10 |
| +6% AME | No | 1.911 | 1985 | 3731 | 4.66 |
| +6% AME | Yes | 1.938 | 2093 | 4009 | 3.17 |
| +6% AME + 12%AA | No | 1.877 | 2151 | 4027 | 1.86 |
| +6% AME + 12%AA | Yes | 1.875 | 2037 | 3726 | 5.66 |
| +6% AME + 12%AA & CP | No | 1.876 | 2063 | 3822 | 3.53 |
| +6% AME + 12%AA & CP | Yes | 1.811 | 2079 | 3655 | 4.88 |

The results set out in Table 27 cover only the starter diets for the initial 21 day period. The results set out in Table 28 cover the whole duration of the routines, that is up to day 42.

From the results set out in these Tables, it can be seen that the addition of the enzyme nearly always has the beneficial effect of reducing the FCR. In addition, it is evident that it is beneficial to add both amino acid and crude protein uplifts to the wheat component of the feeds together with the uplift in AME in order to maximise the response of the broiler chickens to enzyme addition.

The effect demonstrated above of increasing protein value and amino acid values and therefore digestibility of these components of wheat-based diets can also be obtained in feeds based on maize, rye, triticale, barley, sorghum, rice or oats. Further, similar results can be obtained when feeds prepared in accordance with the present invention are fed to other animals such as turkeys, geese, ducks, pigs, sheep and cows.

CLAIMS:

1. Use of a xylanase for assisting livestock to digest protein and/or amino acids present in a feed.

2. Use according to claim 1, wherein the xylanase is included in the feed.

3. Use according to claim 2, wherein the feed comprises 0.00001-10 g/kg of xylanase protein.

4. Use according to claim 3, wherein the feed comprises 0.0001-1 g/kg of xylanase protein.

5. Use according to claim 4, wherein the feed comprises 0.001-0.1 g/kg of xylanase protein.

6. Use according to any preceding claim, wherein the feed comprises at least 20% by weight of a cereal.

7. Use according to claim 6, wherein the feed comprises at least 30% by weight of the cereal.

8. Use according to claim 6 or claim 7, wherein the cereal is at least one of wheat, rye, triticale, barley, oats, sorghum, rice and maize.

9. Use according to claim 8, wherein the cereal is wheat.

10. Use according to any of claims 6-9, wherein the feed further comprises a supplemental source of protein.

11. Use according to claim 10, wherein the supplemental source of protein is fishmeal, meatmeal or a vegetable protein.

12. Use according to claim 11, wherein the supplemental source of vegetable protein is at least one of full fat soybeans, rapeseed, canola, soybean meal, rapeseed meal and canola meal.

13. Use according to any preceding claim, wherein the xylanase is obtained from a fungus.

14. Use according to claim 13, wherein the fungus is *Trichoderma, Aspergillus, Humicola or Neocallimastix.*

15. Use according to any of claims 1-12, wherein the xylanase is obtained from a bacterium.

16. Use according to any preceding claim, wherein the feed comprises at least one further enzyme selected from ß-glucanase, protease, α-amylase and pectinase.

17. Use according to any preceding claim, wherein the livestock is a chicken, a turkey, a goose, a duck, a pig, a sheep or a cow.

18. Use according to claim 17, wherein the livestock is a broiler chicken.

19. A method for enabling the amount of energy and/or protein and/or amino acid supplements included in a cereal-based feed to be reduced without reducing the feed's nutritional value comprising maintaining or increasing the cereal content of the feed whilst adding to it 0.00001-10g of xylanase protein per kg of the feed.

20. A method for increasing the energy and/or protein and/or amino acid nutritional value of the cereal component of a cereal-based feed comprising the step of including 0.00001-10g of xylanase protein per kg of the feed.

21. A method for uplifting the energy and/or protein and/or amino acid value of a cereal-based feed or a component thereof comprising the step of including 0.00001-10g of xylanase protein per kg of the feed.

22. A method according to any of claims 19-21, wherein the cereal-based feed includes at least 20% by weight of the cereal.

23. A method according to claim 22 wherein the cereal-based feed comprises at least 35% by weight of the cereal.

24. A method according to any of claims 19-23, wherein the cereal is wheat.

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Examiner's report to the Comptr ller under Section 17
(The Search report)

| | |
|---|---|
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| | **Date of completion of Search** 16 JUNE 1995 |
| **Databases** (see below) (i) UK Patent Office collections of GB, EP, WO and US patent specifications. (ii) ONLINE: WPI | **Documents considered relevant following a search in respect of Claims :-** 1-24 |

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|---|---|---|---|
| X | GB 2261877 A | (KYOWA) page 3 | 1-3, 6-8, 10-14, 16, 17, 19-23 |
| X | EP 0507231 | (NOVO) page 2 lines 7-8 and page 3 lines 39-49 | 1, 2, 13, 14, 17, 18 |
| X | EP 0463706 A1 | (GIST) page 8 line 56 to page 9 line 8 and Example 8 | 1-11, 13, 14, 17, 19, 24 |
| X | WO 93/25693 A1 | (HAZELWOOD) page 2 lines 1 -13 | 1, 2, 8, 9, 13, 14, 17, 18 |
| X | WO 92/10945 A1 | (ENZYMATIX) Claims 5 and 10 | 1, 2, 16, 17 |
| X | WO 91/04673 A1 | (NOVO) whole document | 1-14, 17, 18, 19-24 |

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